

CLAIMS

1. A method of detecting, and optionally selecting,
5 a DNA sequence, characterized in that the DNA sequence to
be detected possesses a stable expression-enhancing qual-
ity, which method comprises the steps of
 - 1) the cloning in a vector of DNA fragments having a size
of <5000 base pairs between i) a DNA sequence involved
10 in the induction of gene-transcription repressing
chromatin, and ii) a reporter gene comprising a promo-
tor, resulting in a variety of a fragment-comprising
vectors, wherein the distance between the DNA sequence
involved in the induction of the transcription of
15 gene-repressing chromatin and the reporter gene is
fewer than 5000 base pairs.
 - 2) introducing the vectors into host cells, in which host
cells the promotor may be active but induction of the
transcription of gene-repressing chromatin in the
20 vectors results in the repression of the transcription
of the reporter gene; and
 - 3) subjecting the host cells to a selection in order to
identify a host cell exhibiting reporter gene-activ-
ity.
- 25 2. A method according to claim 1, characterized in
that the selection in step 3) occurs by using a reporter
gene which provides resistance to a growth inhibitor and
the host cells are cultivated in the presence of the
growth inhibitor.
- 30 3. A method according to claim 2, characterized in
that the growth inhibitor is present in a concentration
sufficiently high to kill host cells in which the gene
providing resistance to the growth inhibitor is not
active.
- 35 4. A method according to claim 2 or 3, character-
ized in that an antibiotic is used as the growth inhibitor
and the reporter gene is a gene providing resistance to
the antibiotic.

5. A method according to claim 1, characterized in that the reporter gene codes for Green Fluorescent Protein.

6. A method according to claim 5, characterized in 5 that fluorescent host cells are separated from non-fluorescent host cells by means of a Fluorescence-Activated Cell Sorter (FACS).

7. A method according to claim 1, characterized in that the reporter gene is luciferase.

10 8. A method according to ~~any of the preceding~~ claims⁴, characterized in that the fragments have a size of substantially between 2000 - 3000 base pairs.

15 9. A method according to ~~any of the preceding~~ claims⁴, characterized in that the DNA sequence involved with the transcription induction of gene-repressing chromatin is a DNA sequence that is recognized by a heterochromatin-binding protein comprising HP1 which HP1-comprising complex is expressed in the host cell.

20 10. A method according to ~~any of the claims 1 to 8~~, characterized in that the DNA sequence involved with the transcription induction of gene-repressing chromatin is a DNA sequence that is recognized by a complex comprising a Polycomb-group (Pc-G) protein, and the Polycomb-group protein-comprising complex is expressed in the host cell.

25 11. A method according to ~~any of the claims 1 to 8~~, characterized in that the DNA sequence involved with the transcription induction of gene-repressing chromatin is a DNA sequence that is recognized by a complex possessing a histone deacetylase activity, and the histone deacetylase activity-possessing complex is expressed in the host cell.

30 12. A method according to ~~any of the claims 1 to 8~~, characterized in that the DNA sequence involved in the transcription induction of gene-repressing chromatin is a DNA sequence that is recognized by a protein complex comprising MeCP2 (methyl-CpG-binding protein 2), and the MeCP2-comprising complex is expressed in the host cell.

35 13. A method according to ~~any of the preceding~~ claims⁴, characterized in that the DNA sequence involved with the transcription induction of gene-repressing

chromatin is a DNA sequence that is selectively recognized by at least one DNA-binding protein and the organism also expresses a protein complex comprising i) a first part selectively binding the DNA sequence, and ii) a second 5 part inducing the formation of chromatin in which the transcription is repressed.

14. A method according to claim 13, characterized in that the protein complex comprises a fusion protein.

15. A method according to claim 14, characterized 10 in that the first part is a part binding the DNA-binding site of LexA-DNA or GAL4-DNA.

16. A method according to any of the preceding 15 claims, characterized in that the organism in step 1) is selected from the group comprising a plant and a vertebrate.

17. A method according to claim 16, characterized in that the vertebrate is a mammal.

18. A method according to any of the preceding 20 claims, characterized in that the vector is an episomally replicating vector.

19. A method according to any of the preceding 25 claims, characterized in that the vector comprises a replication origin from the Epstein-Barr virus (EBV), OriP, and a nuclear antigen (EBNA1).

20. A DNA sequence selected from i) a DNA sequence 30 isolated from a plant or vertebrate, or derivatives thereof, and ii) a synthetic DNA sequence or one constructed by means of genetic engineering, which DNA sequence is a repression-inhibiting sequence which, by the method according to the present invention can be detected, selected and optionally cloned.

21. A DNA sequence selected from i) a DNA sequence 35 isolated from a plant or vertebrate, or derivatives thereof, and ii) a synthetic DNA sequence or one constructed by means of genetic engineering, which DNA sequence is detected, selected and optionally cloned by means of the method according to any of the claims 18 to 19.

22. A method of making a DNA construct comprising a gene that is to be expressed stably, wherein a stable

expression-promoting DNA sequence is integrated in accordance with claim 20 or 21 in fewer than 2000 bp of the gene.

23. A method according to claim 22, characterized in that the stable expression-enhancing DNA sequence will be integrated both upstream and downstream from the gene.

24. A use of the DNA construct obtained in accordance with claim 22 or 23, wherein the DNA construct is a vector for the transformation of an organism.

Add (B)